

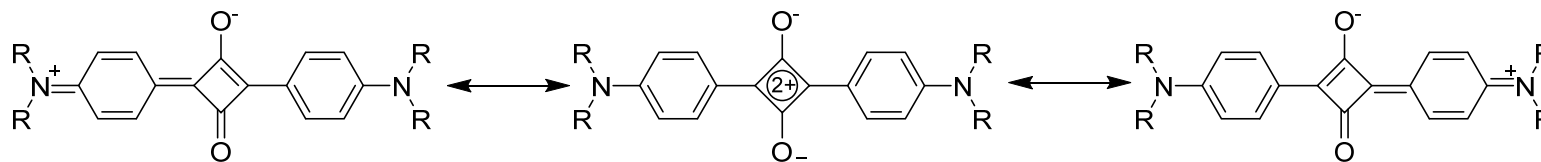
# AMINONAPHTHALIMIDE-SQUARINE DYAD: PHOTOPHYSICAL PROPERTIES AND BIOIMAGING APPLICATION



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## Squaraine fluorophore

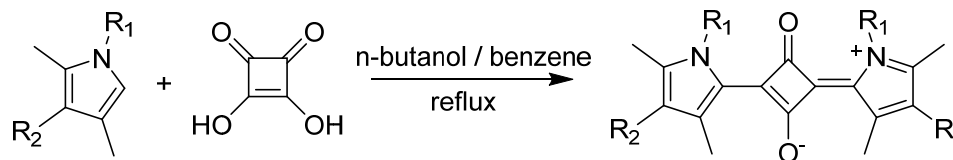


*Donor*

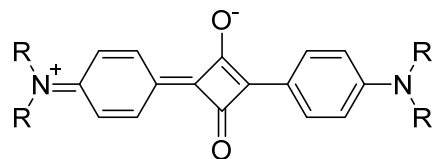
*Acceptor*

*Donor*

Treibs and Jacob (1965)

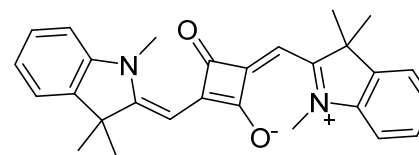


Sprenger and Ziegenbein (1966)



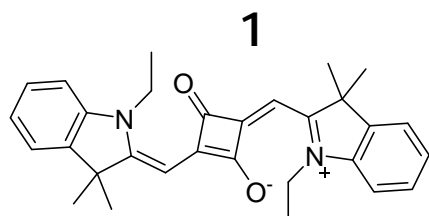
Aromatic squaraine

Sprenger and Ziegenbein (1967)

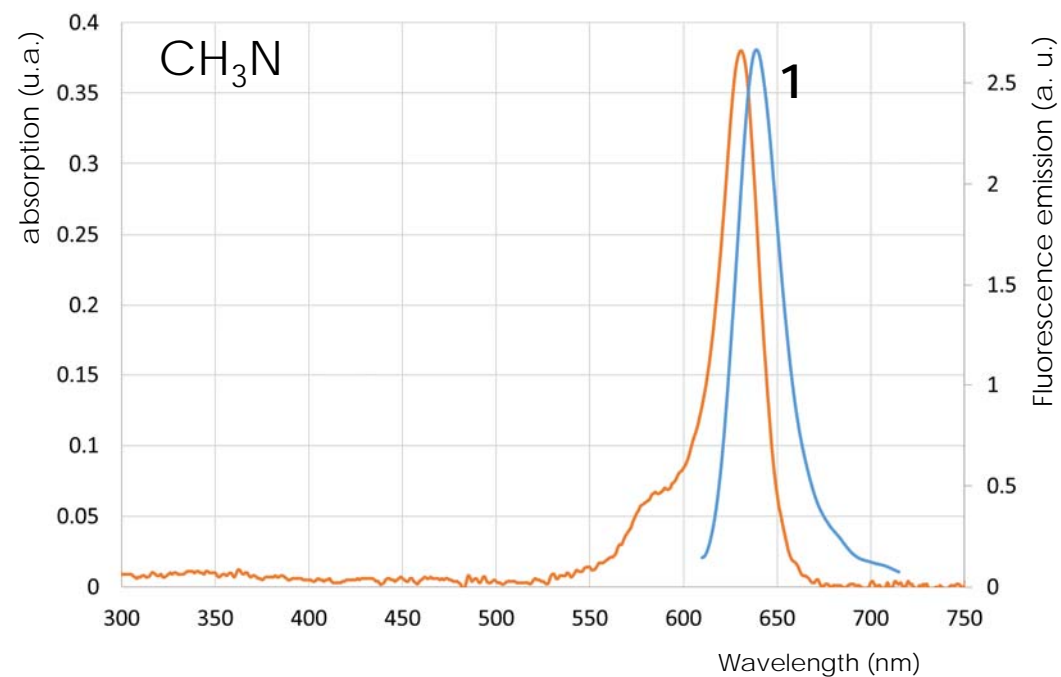


Heterocyclic squaraine

## Squaraine fluorophore

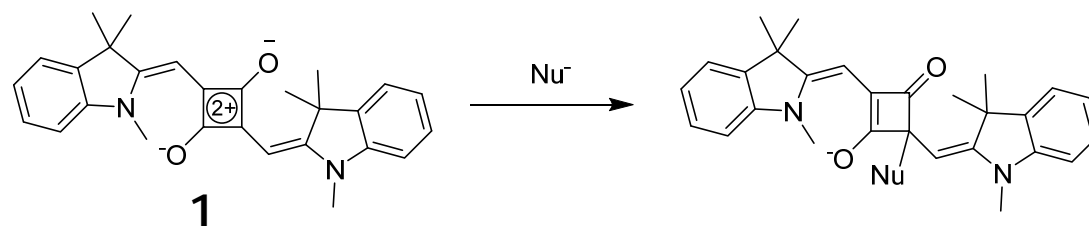


Heterocyclic squaraine



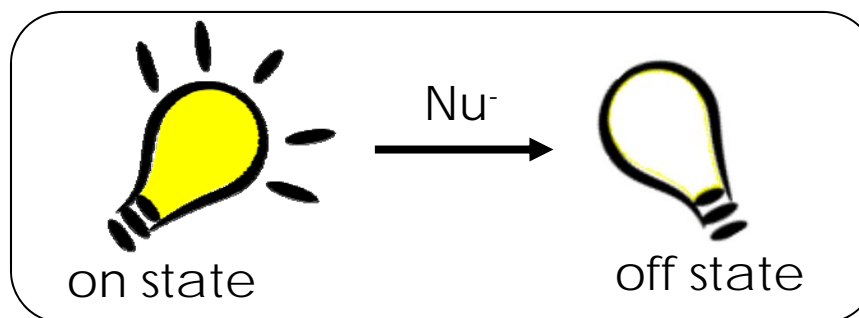
Solvent	$\lambda_{\text{abs max}}$ (nm)	$\epsilon$ ( $\times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ )	$\lambda_{\text{em max}}$ (nm)	$\Phi_{\text{FL}}$
CH <sub>3</sub> CN	638	2.40	642	0.12
Toluene	641	3.28	651	0.38

## Squaraine fluorophore: interaction with nucleophiles



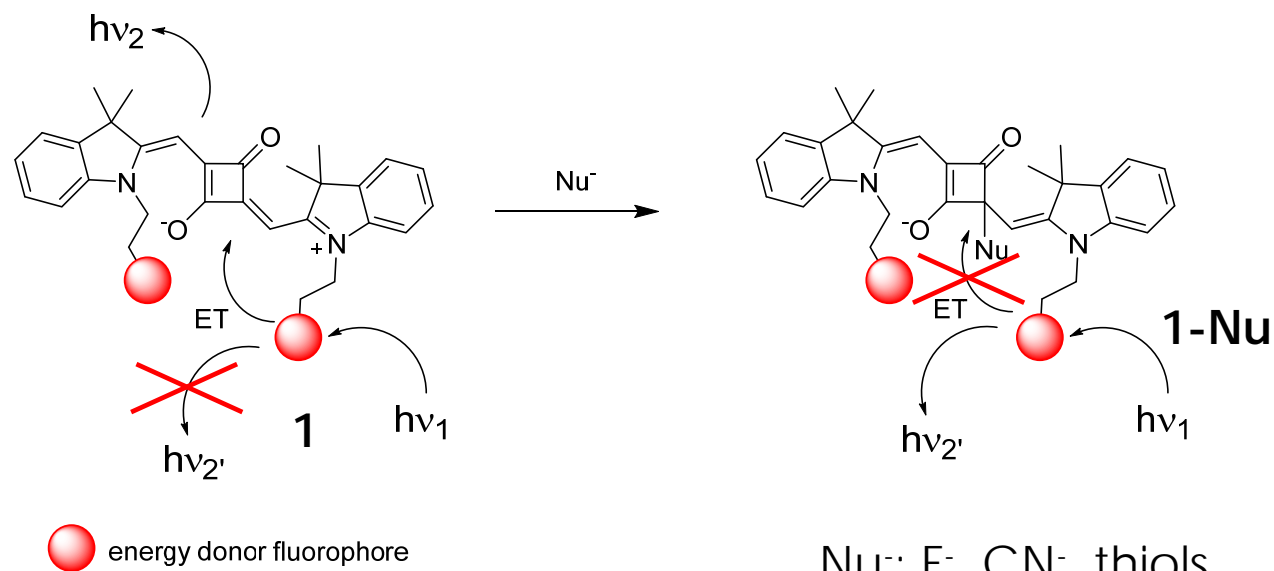
Heterocyclic squaraine

$\text{Nu}^-$ :  $\text{F}^-$ ,  $\text{CN}^-$ , thiols

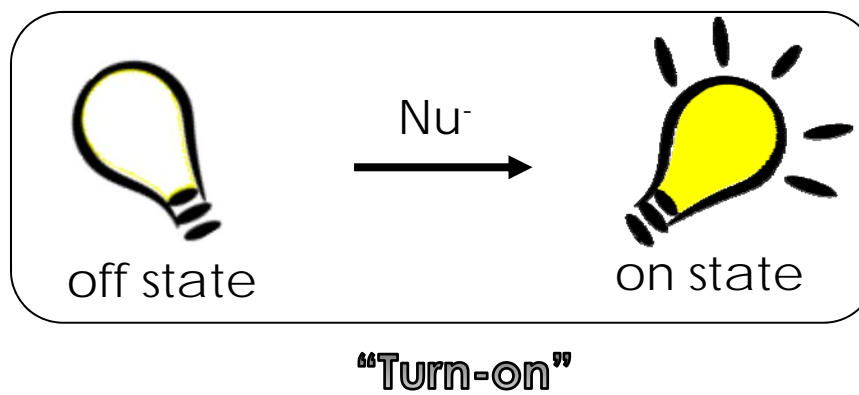


“Turn-off”

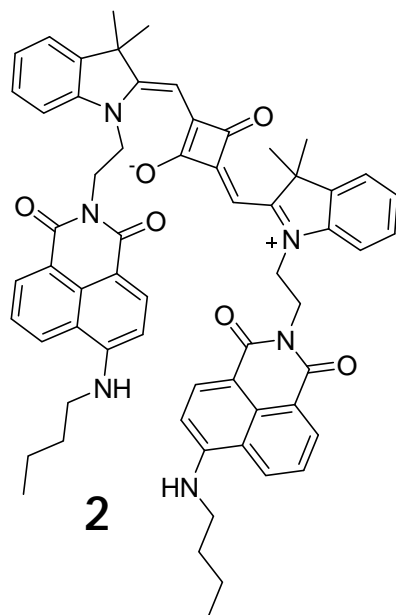
## Squaraine fluorophore: interaction with nucleophiles



$Nu^-$ :  $F^-$ ,  $CN^-$ , thiols



## 4-Amino-1,8-naphthalimide-squaraine-dyad

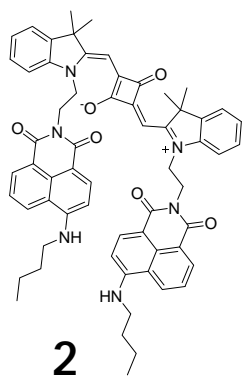
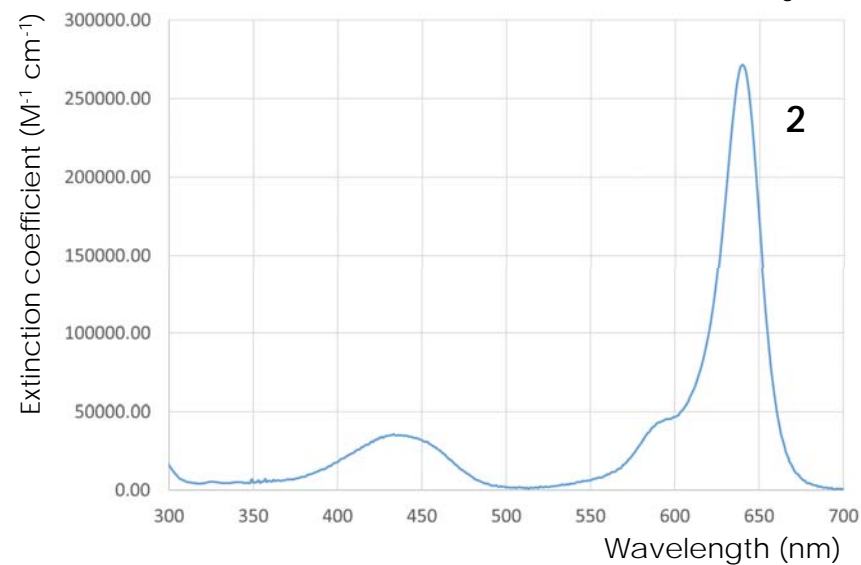
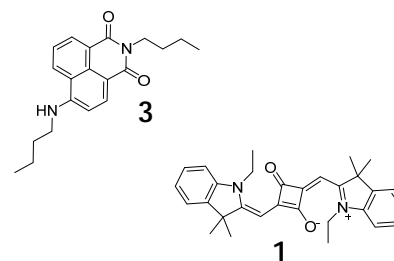
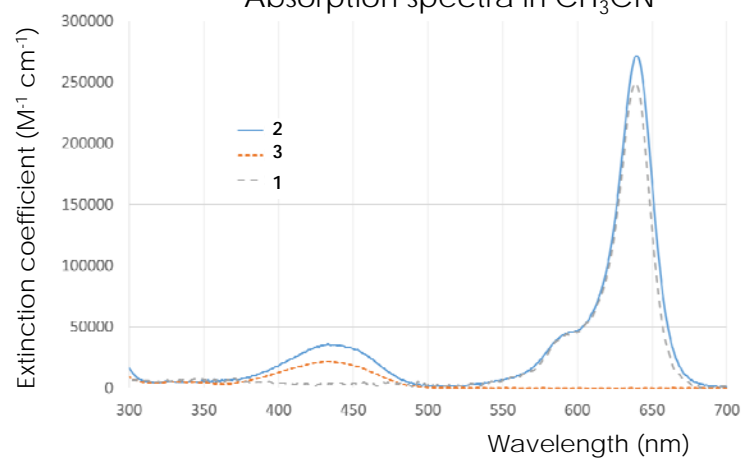


Energy Transfer Cassettes

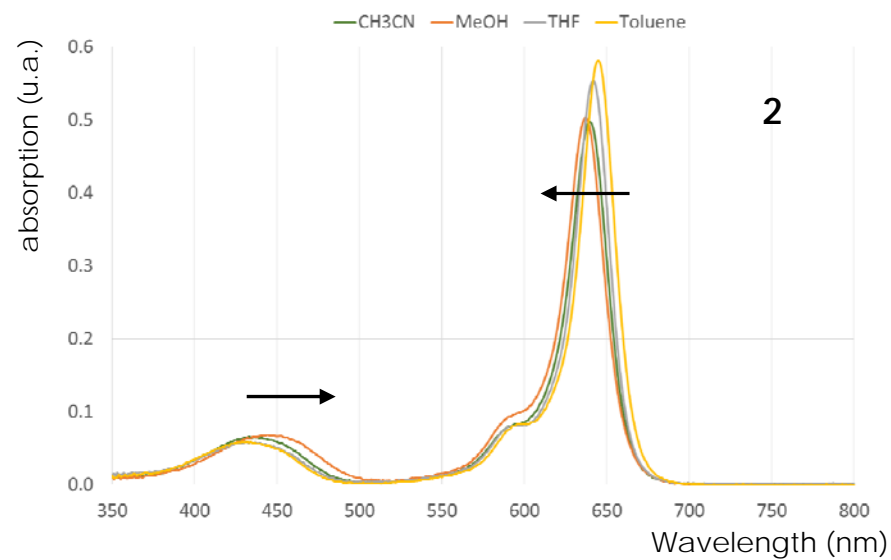
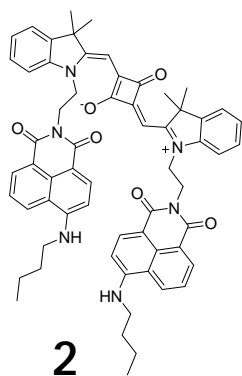
**ACCEPTOR**  
Fluorescent entity

**DONOR**  
Fluorescent entity

naphthalimide-squaraine-dyad

Absorption spectrum in CH<sub>3</sub>CNAbsorption spectra in CH<sub>3</sub>CN

# naphthalimide-squaraine-dyad

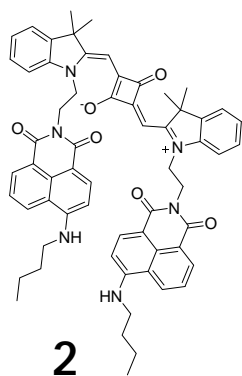


Absorption spectra of **2** (1,8  $\mu$ M) in different solvents

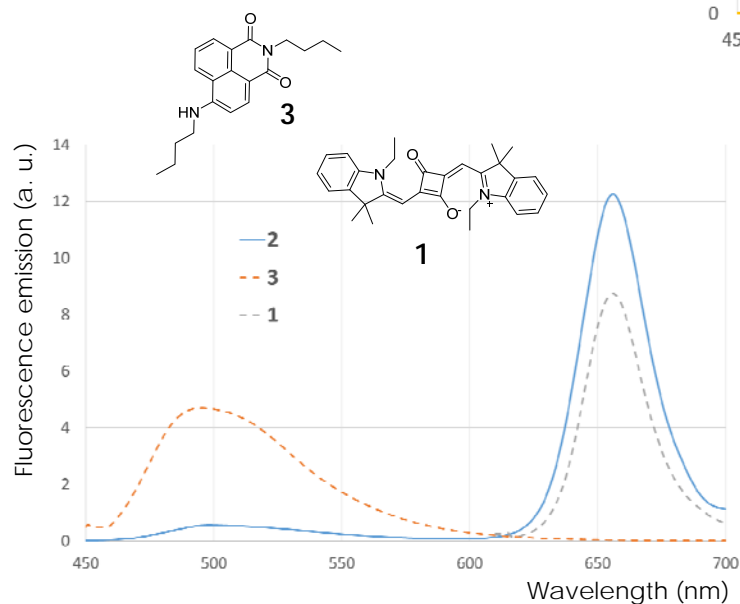
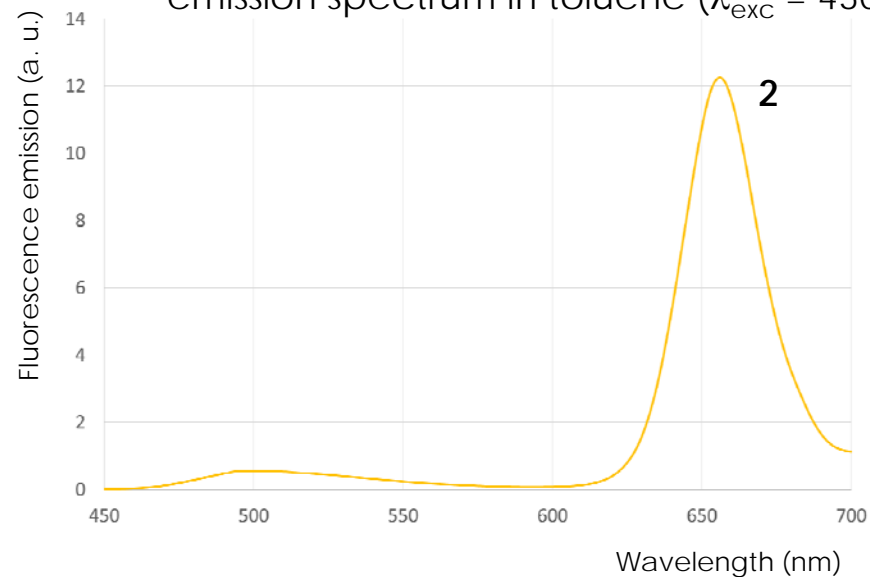
Solvent	$\lambda_{\text{naphthalimide}}$ (nm)	$\lambda_{\text{squaraine}}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )
MeOH	446	637	2.64
CH <sub>3</sub> CN	437	639	2.71
THF	433	642	3.03
Toluene	433	646	3.18



# naphthalimide-squaraine-dyad



emission spectrum in toluene ( $\lambda_{\text{exc}} = 430 \text{ nm}$ )



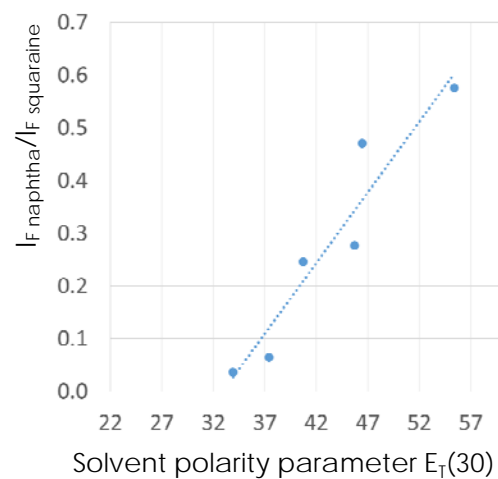
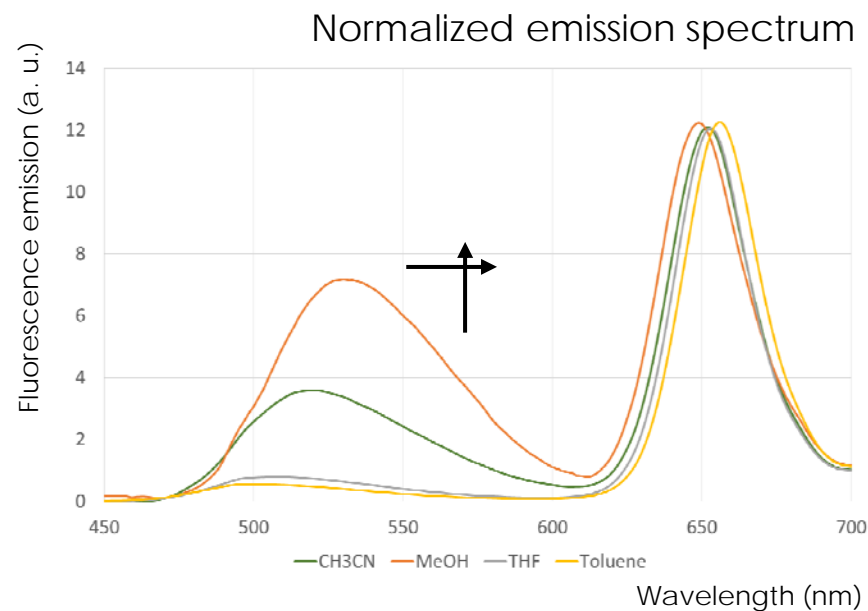
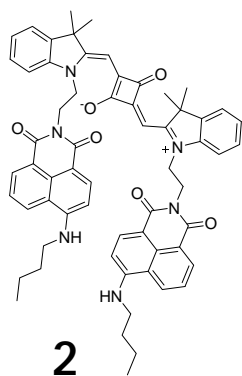
Calculation data of energy transfer in toluene

$$R_0 = 20.1 \text{ \AA}$$

$$J = 1.96 \times 10^{-11} \text{ cm}^6 \text{ mol}^{-1}$$

$$\Phi_{\text{EET}} = 0.985$$

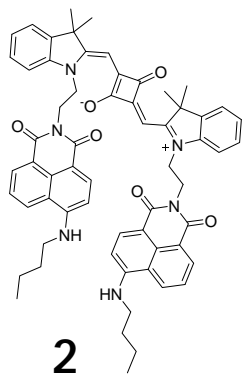
# naphthalimide-squaraine-dyad



Emission spectra of **2** (1,8  $\mu$ M) in different solvents

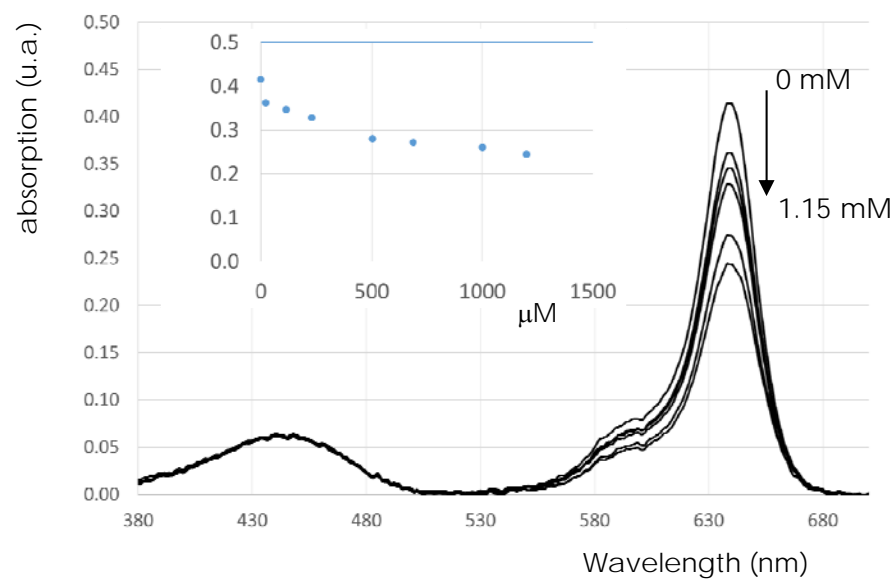
Solvent	$\lambda_{\text{naphthalimide}}$	$\lambda_{\text{squaraine}}$	$\Phi$	$I_{F \text{ naphtha}} / I_{F \text{ squaraine}}$
MeOH	532	650	0.06	0.58
CH <sub>3</sub> CN	521	652	0.15	0.28
THF	506	653	0.27	0.07
Toluene	503	656	0.31	0.04

# naphthalimide-squaraine-dyad

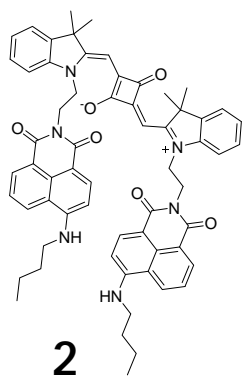


Effect of nucleophiles: NaCN

Absorption spectrum of **2** (2,0  $\mu\text{M}$ ) in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  (9:1)



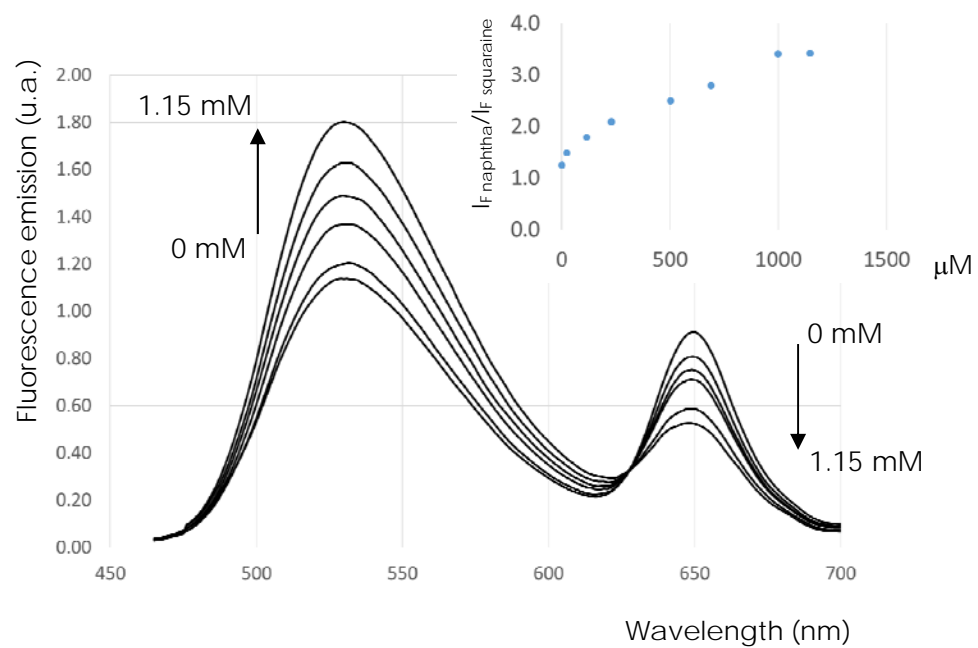
# naphthalimide-squaraine-dyad



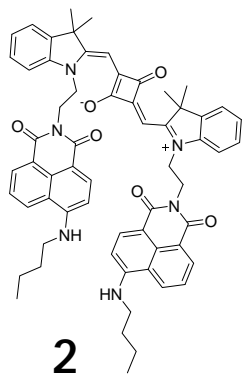
( $\lambda_{\text{exc}} = 440 \text{ nm}$ )

## Effect of nucleophiles: NaCN

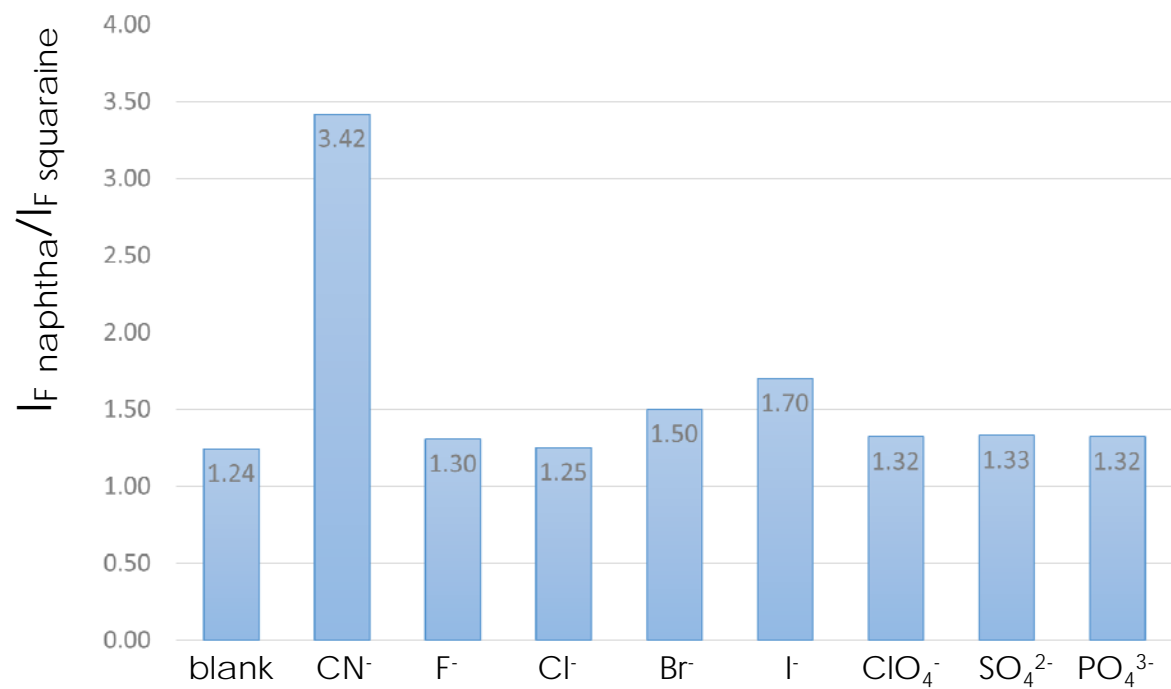
Emission spectrum of **2** ( $2.0 \mu\text{M}$ ) in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  (9:1)



# naphthalimide-squaraine-dyad



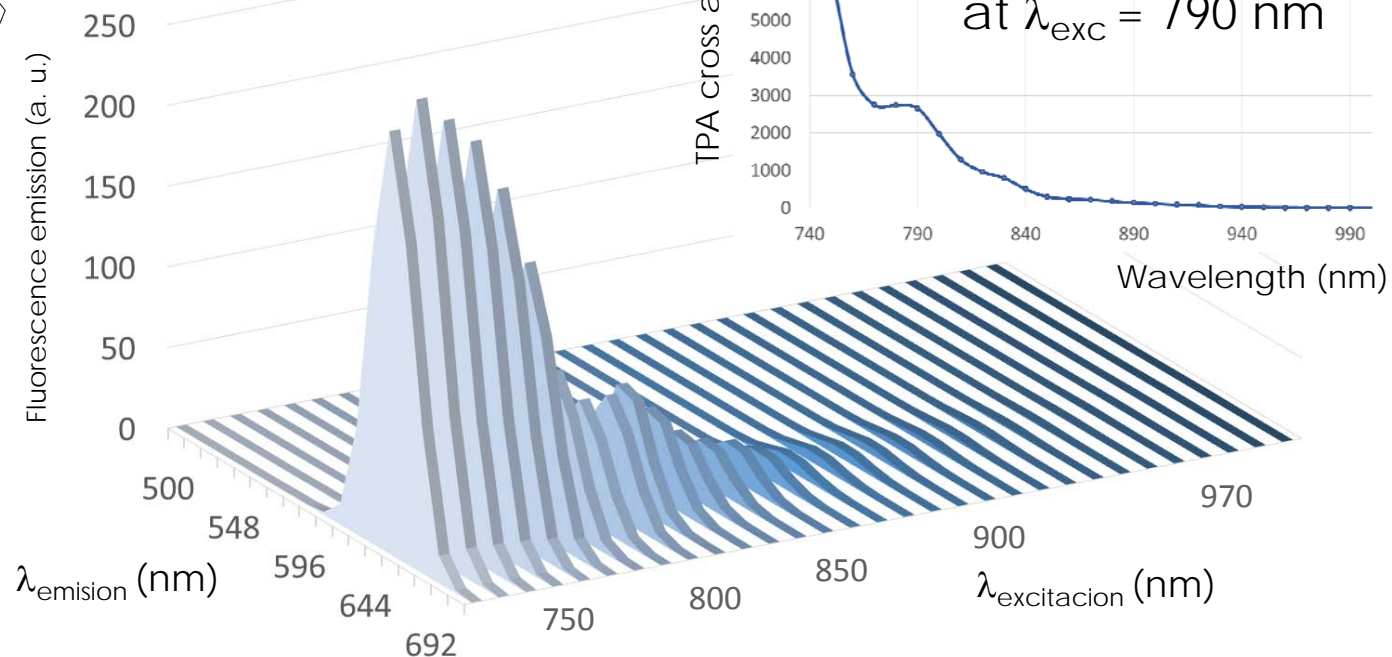
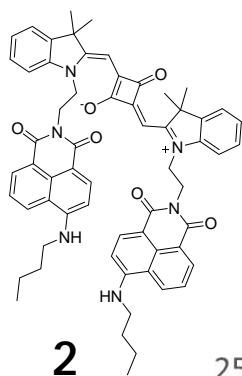
## Effect of nucleophiles



Maximum emission spectra change in presence of some anions  
in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  (9:1)

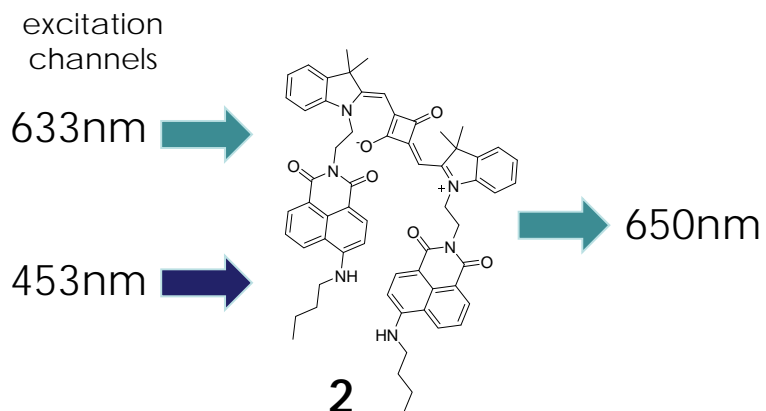
# naphthalimide-squaraine-dyad

## Two photon emission

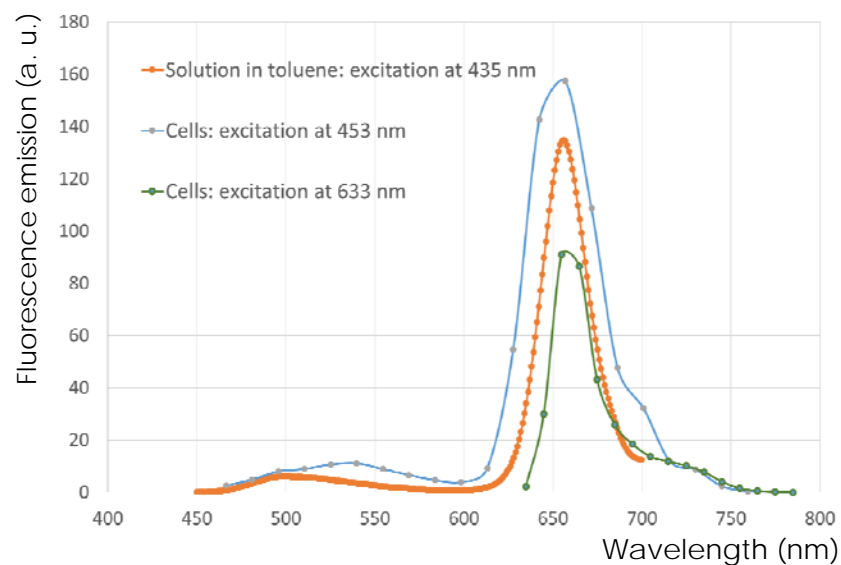
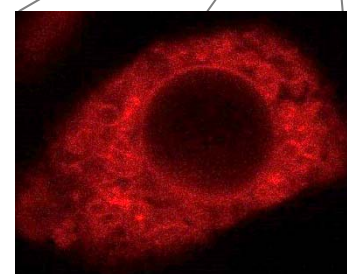
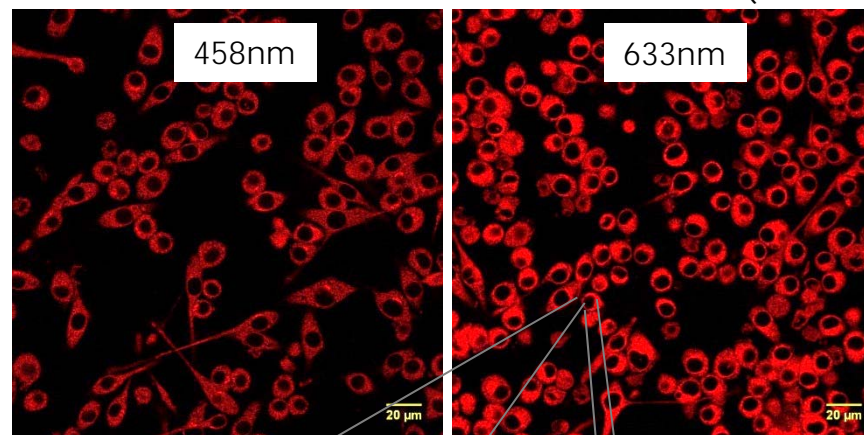


Two photon emission spectra of **2** (2.6  $\mu\text{M}$ ) in toluene

# One photon fluorescence microscopy imaging



Confocal microscopy images of N13 microglial cell line incubated with **2** ( $10^{-7}$  M)

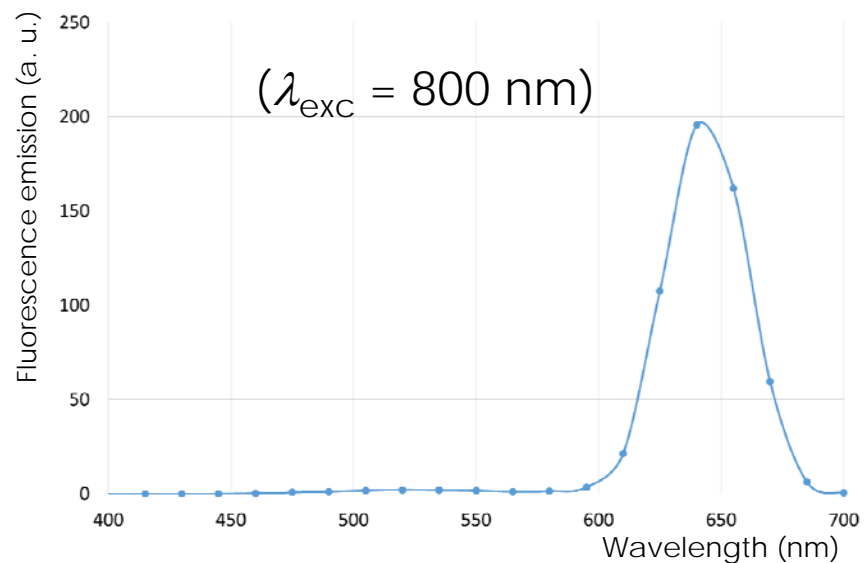
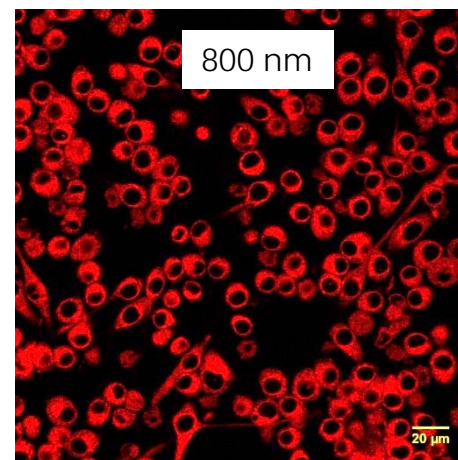
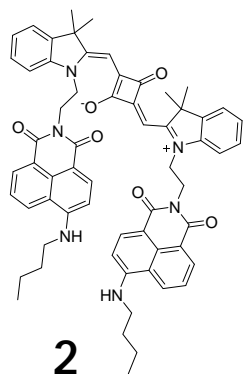


Confocal fluorescence emission

$$\text{Cells: } I_{F \text{ nafta}} / I_{F \text{ squaraine}} = 0.07$$

## Two-photon fluorescence microscopy imaging

Two photon microscopy images of N13 microglial cell line incubated with **2** ( $10^{-7}$  M)



Two photon fluorescence:

$$I_{F \text{ nafta}} / I_{F \text{ squaraine}} = 0.01$$

Confocal fluorescence:

$$I_{F \text{ nafta}} / I_{F \text{ squaraine}} = 0.07$$





# Thanks you for your attention



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**Ministerio de Economía y Competitividad (Spain)**



**Consejería de Salud, Junta de Andalucía (Spain)**

